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Managing aquatic parasites for reduced drug resistance: lessons from the land

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Atlantic salmon farming is one of the largest aquaculture industries in the world. A major problem in salmon farms is the sea louse ectoparasite *Lepeophtheirus salmonis*, which can cause stress, secondary infection and sometimes mortality in the salmon host. Sea lice have substantial impacts on farm economics and potentially nearby wild salmonid populations. The most common method of controlling sea louse infestations is application of chemicals. However, most farming regions worldwide have observed resistance to the small set of treatment chemicals that are available. Despite this, there has been little investigation of treatment strategies for managing resistance in aquaculture. In this article, we compare four archetypical treatment strategies inspired by agriculture, where the topic has a rich history of study, and add a fifth strategy common in aquaculture. We use an agent-based model (ABM) to simulate these strategies and their varying applications of chemicals over time and space. We analyse the ABM output to compare how the strategies perform in controlling louse abundance, number of treatments required and levels of resistance in the sea louse population. Our results indicated that among the approaches considered applying chemicals in combination was the most effective over the long term.

1. Introduction

Evolution of chemical resistance is a challenge that comes hand-in-hand with using pesticides and drugs to control unwanted taxa. Repeated use of lethal chemicals places strong selection pressure on exposed populations and, where there is heritable genetic variation for resistance, this can lead to the evolution of chemical resistance that results in the decreased efficacy of chemical controls. For example, pesticide resistance is a well-known issue in terrestrial environments, where in North America more than 250 species of terrestrial arthropods are resistant to at least one chemical pesticide [1]. The decreased ability to control pests and pathogens has economic, environmental, public health and food security challenges [2–4]. Recognizing the scale of this problem, recent papers have called for more investigation into strategies that simultaneously manage pests and pathogens and their evolutionary trajectories [4,5].

Strategies for reducing the evolution of pesticide resistance in terrestrial systems involve changing patterns of chemical applications in both time and space [3–5]. The goal of these strategies is to increase the degree of treatment heterogeneity (DTH). DTH is defined by the REX Consortium [5] to be 'the probability that a set of resistance genes is confronted by more than one pesticide or drug within or between generations'. In theory, the evolution of treatment resistance is slower when the DTH is higher. This occurs because target pests are exposed to more than one type of selection pressure due to a chemical application, thus diluting the strong selection a single type would incur. In most terrestrial cases, 'combination' treatments, where pests are hit simultaneously

with more than one chemical, are the most efficient in removing pests while having relatively slower evolution of resistance [3,5,6]. Combination treatments typically have a high DTH relative to other approaches where chemicals may be rotated over time, or applied in spatial mosaics [5].

By contrast to terrestrial systems, pesticide resistance is a more recent problem in aquatic environments [7–10] where high-intensity farming of single species (i.e. modern aquaculture) and reliance on chemical control of pests are recent developments [11,12]. In aquatic systems, the dispersal, growth and development of many pests are all strongly tied to environmental conditions, particularly hydrodynamic flow and temperature, which vary in time and space on several scales [13,14]. We are unaware of any studies that have examined how typical resistance strategies for terrestrial systems function to control pests and their evolutionary trajectories in marine systems.

Sea lice, crustacean ectoparasites of salmon, are one of the most persistent and damaging causes of losses to the salmon farming industry—one of the largest aquaculture industries in the world [15]. When they end their planktonic life stages, these copepods attach to the epithelium of their salmonid hosts and can cause stress, secondary infections and occasional mortality in their hosts; negatively impacting profitability, farm output and, in some cases, adversely affecting nearby wild salmonid populations [15–17]. Resistance to a number of commonly used chemical treatments has already evolved in sea lice in most major salmon producing areas including Norway, Scotland, the Faroe Islands, Ireland, Chile and eastern Canada (reviewed in [10]). Owing to the limited types of chemical treatments available for salmon infected with sea lice, many salmon farmers are running out of chemical options for effective control [7,8,10,18]. As only a few new active compounds for controlling sea lice have been developed during the past 30 years [19], it is imperative to maintain the efficacy of the existing compounds by using appropriate management techniques.

Agent-based models (ABMs) are an ideal yet underused tool for examining resistance evolution (e.g. [18,20]). These inherently stochastic models, which model processes at the individual level and observe emergent behaviour at higher order levels, are particularly well suited for investigating evolutionary processes where selection occurs at the level of the individual, yet it is the population that evolves [21]. Factors that are historically under-represented in more traditional resistance models, such as spatial structure, use of multiple chemicals and cross-resistance, can be easily incorporated into ABMs [22]. In addition, studying evolutionary processes in non-model systems using empirical approaches is slow, may not be ethical (i.e. if it leads to outbreaks of pests in the wild) and may be subject to unquantifiable outside influence (e.g. migration, extreme disturbances causing bottlenecks, etc.). In such cases, simulations are the only feasible approach to understanding resistance evolution.

In this paper, we develop an ABM of sea lice and their salmonid hosts that we use to examine how to strategically manage sea louse infestations while at the same time minimizing opportunities for resistance evolution in hypothetical populations. We evaluate the effectiveness of various 'terrestrial' strategies in controlling pests and compare their relative merits in controlling sea louse infestations and retarding the rate that genetic resistance evolves.

2. Material: model

To investigate the relative merits of different treatment strategies in an Atlantic salmon farming context, we built an ABM using the AnyLogic™ modelling software (www.anylogic.com).

Our model was modified from the model described in [18]. Both models evaluate sea louse infestations on populations of farmed fish and the evolution of chemical resistance in response to treatments. The initial model was used to investigate interactions between farmed and wild fish. The current model, which is focused on the strategic use of multiple chemicals, does not include wild salmonids but does have greater capabilities for defining and strategically applying sea louse treatments.

Following the lead of [18], we use 'sea louse agents' to refer to modelled sea lice, and 'sea lice' to refer to real sea lice. Below, we use the overview, design and details protocol [23] to further describe the modifications made for this model. Likewise, all terms below that are preceded by the word 'agent'—e.g. 'agent fitness'—refer to concepts in the model rather than biological terms.

We use the terms 'strategy' and 'scenario' to have similar, but distinct, meanings. A strategy is an approach to making use of the available chemicals; for example, a strategy would be to use combination treatments (as described previously) whenever a threshold number of sea lice is exceeded. A scenario is a particular model configuration. Because we are comparing treatment strategies in this study, there is a scenario for each strategy.

2.1. Overview

The ABM simulates *Lepeophtheirus salmonis* infestations on an Atlantic salmon farm. The farm is stocked with young salmon that are held in the farm until harvesting. The farm is then fallowed for a time, before re-stocking. At each re-stocking point, there are no sea lice on the farm or on the fish. However, there is an external flow of planktonic lice onto the farm that can infest the salmon. The sea lice attach to the salmon, breed and produce eggs that hatch into new sea lice. The salmon farmer in the simulation has two chemicals, both based on bath application, available to control sea louse numbers. In this study, we compare different strategies of using these chemicals to manage sea louse infestations on the farm.

2.1.1. Purpose

The purpose of this model is to simulate an Atlantic salmon farm with sea louse infestations, and investigate the effects of different treatment strategies on resistance evolution. We compare four different strategies that represent archetypical approaches in terrestrial systems [5], and add one of our own. We also include two variations of two of the strategies, for a total of seven. We compare these seven strategies in terms of their ability to control sea louse infestations and the evolution of resistance to the chemical treatments.

2.1.2. State variables and scales

Salmon agents and sea louse agents are grouped into two shared communities within a farm. To allow for spatial heterogeneity in the application of treatments, the farm is divided into two cages (i.e. 'communities'), which can

receive chemical treatments independently and may exchange planktonic sea lice.

We model salmon and sea louse life histories as in [18]. However, sea louse genetics are more complex due to the addition of multiple chemical treatment types.

In each scenario, we ran the model for 12 years, consisting of six 2 year farm cycles. Each cycle is made up of 656 farming days and 74 days of fallowing. This time period is on the same scale that resistance has been observed to develop in a number of salmon farming areas [24–26]. Time is modelled continuously.

2.1.3. Process overview and scheduling

Stocking, harvesting and fallowing occur on a 2 year cycle. Annual temperature patterns are modelled with a sine curve (see below). As the aim of this study is to investigate different treatment strategies, we have extended the model to be able to apply multiple types of chemotherapeutants in different temporal and spatial patterns.

There is a temperature-scaled external flow of sea louse agents into the farm. The temperature dependency reflects the slower production of sea lice in the winter [27] and the number of arriving sea louse agents is calculated as

$$\frac{\text{number of salmon hosts} \times \text{temperature}}{10}.$$

The resistance of these arriving sea louse agents is the same as the average resistance of the sea louse agents on the farm on the day that they arrive.

2.2. Design concepts

The design concepts are mostly the same as the initial model, with the following modifications.

2.2.1. Agent fitness

We have extended the model with the option to apply two independent chemical treatments. We model resistance to each chemical separately by giving each sea louse agent two genes (one for each chemical), each of which has two codominant alleles, one of which confers resistance. There is no cross-resistance to the two chemicals. We have used single gene resistance as our logical starting point in the model. This is based on azamethiphos resistance, which is the best described resistance mechanism for sea lice [28]. Multi-gene and epigenetic resistance mechanisms, though potentially common [29,30], require understanding of the interactions of multiple genes at pre- and post-translational levels, increasing complexity and requiring a more complete understanding. A third 'neutral' gene, which has no effect on fitness, is modelled in order to understand how the model structure and its inherent stochasticity may be affecting population genetic structure.

2.2.2. Agent collectives

The farm is now divided into two cages. Each cage contains salmon agents, attached sea louse agents on the salmon agents and planktonic sea louse agents in the water column. Sea louse agents are able to attach to salmon agents in another cage with a parameter-defined probability.

2.3. Details

2.3.1. Initialization

The farm is initialized with newly stocked salmon agents on 1 May (day 120 in the temperature cycle). These salmon agents are evenly divided between the two cages. There are no sea louse agents on the farm at initialization.

2.3.2. Input

We adapted a temperature profile from [27] for our simulations. This profile was derived by fitting a sine curve to daily temperature data from 33 salmon farms across 5 years in Scotland [31]. In our model, we raised the temperature by 1°C in order to increase sea louse infestation rates and accelerate the rate that resistance (our variable of interest) evolves. The new temperature function, which results in 1°C higher temperatures than in the previous model, is shown in equation (2.1). This function was used in all scenarios.

$$\text{temperature} = 6.19\left[\sin\left(\frac{3.14 \times (\text{day} + 58)}{365}\right)\right]^2 + 8.07. \quad (2.1)$$

Equation (2.1) is the calculation for water temperature on a particular day.

2.3.3. Submodels

Multiple cages. The connectivity of sea louse agents between the two cages is controlled by a model parameter. Sea louse agents arriving externally have equal probabilities of ending up in either cage, while those that hatch on the farm are assigned to the same cage as the mother. When attaching to a host, the connectivity parameter defines the probability of the planktonic sea louse agent attaching to a host (salmon agent) in another cage versus the same cage. Adult males, which frequently switch hosts in order to find mates [32], switch cages with the same probability as planktonic sea louse agents. The relationships between cages, including external flow, attachment, cross-flow of sea lice agents and salmon agent allocation, are shown in figure 1.

Treatments. We have extended the treatment capabilities of the model to allow us to simulate the different treatment strategies in the model scenarios. There are three treatment extensions. First, for each chemical type, there are model parameters to specify efficacy at killing each sea louse developmental stage, the effect of each allele on resistance and the fitness cost of resistance (if any).

Second, the minimum number of days between treatments can be adjusted in order to reflect varying schedules for treatments and counting sea lice. We also specify a number of days before harvest when treatments cannot be applied, as a withdrawal period is often mandated in order to reduce the amount of residual chemicals in a market fish [33,34].

Third, there is greater flexibility in specifying when and which chemicals are applied at any time. Treatments can be triggered in two ways—when the count of lice per salmon exceeds a threshold (as typically occurs), or at a particular scheduled time (which might occur prior to a wild salmon migration, for example). If the treatment is based on a threshold, attached sea lice are counted weekly and a treatment is applied if the threshold is exceeded. If the treatment is scheduled, it will be applied on that Julian day for every stock–harvest–fallow cycle.

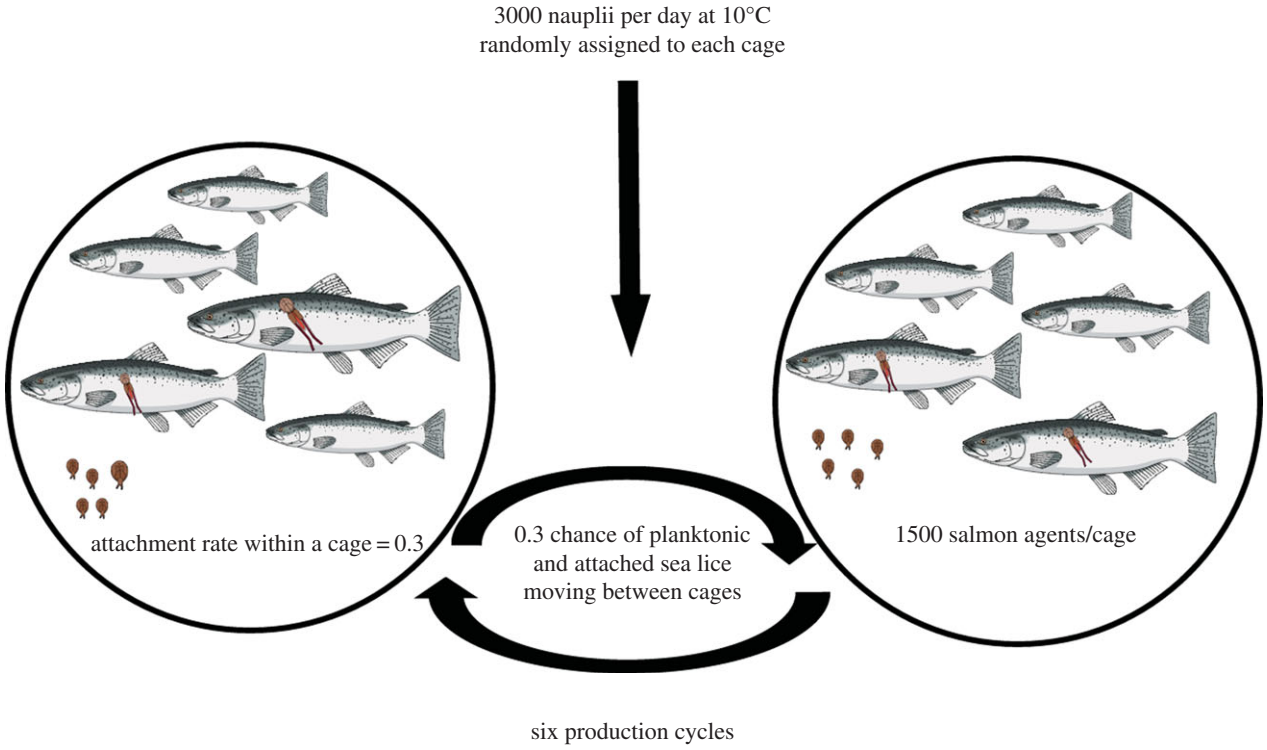

Figure 1. Some of the submodels in the modelled farm are shown. The farmed salmon are evenly split between two cages. There is an external flow of sea louse nauplii that are evenly distributed to the cages. Sea lice on the farm can attach to hosts, with an associated probability of success, and a probability of switching cages while doing so. (Online version in colour.)

In addition, after it has been determined that a chemical is to be applied, the model can be configured to select between the available chemicals in different ways. There are three options: (i) apply a subset of the available chemical types each time; (ii) rotate through a list of chemical types, changing each time; and (iii) rotate through a list of chemical types, changing when the current type fails to control sea louse infestations. With each of these options, it is possible to restrict the treatment to a subset of the cages.

3. Methods: scenarios

We used the described model to simulate and compare five strategies (with two variations for seven total) for applying chemical treatments to control sea lice. The first four are based on agricultural strategies as summarized by the REX Consortium [5], and we added the fifth—rotation—to reflect a common ad hoc strategy commonly employed by Atlantic salmon farmers. We configured a model scenario for each of these strategies. In all scenarios, we stocked 3000 salmon agents, distributed evenly between two cages. The minimum inter-treatment duration was 14 days and no treatments were permitted for the last 60 days of a response cycle. We ran 150 iterations of each scenario.

The five strategies are:

1. Responsive alternation. This strategy is to use one chemical for each treatment event until resistance develops, at which time a second chemical is used. This approach often occurs when there is no resistance management.
2. Periodic application. Chemical treatments are applied on a time schedule, regardless of infection status. The chemical used is alternated with each treatment. There are two variations of this strategy: in the first, the time between applications is shorter than the parasite generation time, and, in the second, the time between applications is longer than the generation time. Both will probably lead to frequent and unnecessary treatments on a salmon farm. We include them unmodified for completeness in our comparison with terrestrial strategies.
3. Mosaic application. In this strategy, treatments are applied according to a spatial pattern with distinct regions with non-overlapping boundaries. At least two chemicals are applied simultaneously to different regions. There are also two variations of this strategy: in the first, regions do not exchange parasites, and, in the second, there is some dispersal between the regions. While this is common in agriculture, it is not explicitly used in aquaculture. However, treating cages at different times could result in an implicit use. Both variations could occur on salmon farms depending upon the hydrodynamics of the site [35].
4. Combination application. This is the use of two or more chemicals across both time and space. All available chemical types are applied simultaneously to all areas at each treatment event.
5. Rotation. In this strategy, the farmer uses a different chemical for each treatment event. When all available chemical types have been used, they start back with the first chemical used.

Including the two variations of mosaic and periodic application, we examined seven scenarios in total. In all seven scenarios, we used two chemical types. Both chemical applications were designed to simulate instantaneous bath treatments (as opposed to longer 'in-feed treatments'), and each chemical had a 95% efficacy against all attached sea louse agents in the absence of resistance (i.e. chalimus, pre-adult and adult stages). Both chemicals had an associated

Table 1. Details of the seven scenarios. C1 = chemical 1; C2 = chemical 2. All scenarios have two cages.

treatment strategy	summary	treatment trigger/schedule	connectivity of cages
responsive alternation	start with C1. Switch treatment type whenever the current type fails (defined if efficacy of treatment is less than 40%)	two adult sea lice/salmon	30%
periodic application—within generation	alternate between C1 and C2. Treatments are scheduled such that inter-treatment duration is less than the generation time	days: 11, 55, 91, 124, 158, 196, 244, 308, 368, 413, 449, 483, 516, 552	30%
periodic application—longer than generation	alternate between C1 and C2. Treatments are scheduled such that inter-treatment duration is greater than the generation time	days: 11, 77, 128, 179, 243, 340, 418, 472, 521, 578	30%
rotation	alternate between C1 and C2	two adult sea lice/salmon	30%
mosaic—no dispersal	C1 is used exclusively in cage 1 and C2 is used exclusively in cage 2	two adult sea lice/salmon	0%
mosaic—with dispersal	C1 is used exclusively in cage 1 and C2 is used exclusively in cage 2	two adult sea lice/salmon	30%
combination	C1 and C2 are applied simultaneously to all cages	two adult sea lice/salmon	30%

resistance benefit of 45%, which is the reduction in chemical efficacy provided by each resistant allele: i.e. a fully susceptible attached sea louse agent had a 95% chance of dying during treatment, a heterogeneous agent had that chance reduced by 45% and a fully resistant agent with both alleles present had its chance of dying reduced by 90%. There was no cross-resistance from one chemical to another. Table 1 shows the details of how treatments were applied in each of the scenarios.

We added a small fitness cost of resistance to one of the chemicals in order to improve the presentation of our results. Sea lice with this codominant allele had a 1% decrease in fecundity for each allele they possessed. Without this fitness cost added, the resistance to each chemical was identical and the trends were challenging to visualize. The small fitness cost that we added serves to differentiate them without qualitatively changing the results.

While the application of treatments in response to a threshold was fairly straightforward (i.e. apply if the threshold count is exceeded), the timing of pre-scheduled treatments required more analysis. To determine the timing of the periodic application scenarios, we used a numerical approximation of development times based on the current temperature. Starting at the day of the most recent treatment, each day we calculated the average progress in a developmental cycle at the temperature for that day and added to a running total. When we reached 100%, we marked the stage as developed and started calculating the next development stage. For example: we started calculating average chalimus development on day 11 when the temperature was 10.007°C and the average progress in the chalimus stage was 5.47%; on day 12 it was 10.056°C so chalimus progress developed an additional 5.50%, for a total of 10.97% over these 2 days; by day 29, the development total had reached 100% so we started calculating the average time to develop the pre-adult stage; the generation time included the chalimus, pre-adult, 1 day to extrude egg strings and the time for eggs to hatch.

In both the periodic scenarios, the first treatment in a farming cycle was scheduled after the time taken to progress through nauplii and copepodid stages (11 days), as the only sea lice to arrive were planktonic. After that we used time between attachment to hatching of first clutch as the development time. To determine times for within generation treatment, we first calculated the full generation time, and then multiplied that by 0.8 to get the next treatment time. The 'longer than generation' treatment times were calculated by multiplying by 1.2. The scheduled times are shown superimposed on the temperature curve in figure 2.

4. Results and discussion

In each of the seven scenarios, we recorded sea lice abundance, number of treatment events and resistance levels. To investigate the relative strengths and weaknesses of the strategies involves all three of these interrelated measures. While sea louse abundance is the final indicator of the parasite's impact on farm output, chemotherapeutants are expensive so the number of treatment events applied is economically important to the farmer, and the rate of evolution of resistance indicates how effective chemical control will be in the long term.

In this section, we start by comparing the seven scenarios with regards to our three measures—abundance, treatments and resistance. In the main figures, we show only the *responsive*, *mosaic with dispersal*, *rotation*, both *periodics* and the *combination* scenarios.

We then discuss the bigger picture and broader implications of this work.

4.1. Sea lice abundance levels

In figure 3, we show the abundance of adult male sea louse agents over six production cycles (electronic supplementary material, table S1 shows the average abundance for all attached life stages in all scenarios, as well as the sum of

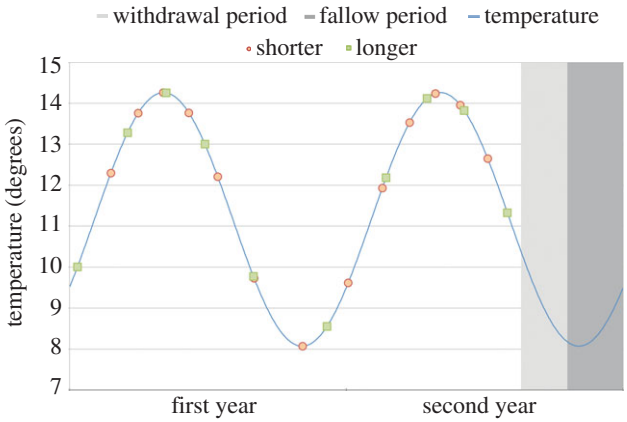

Figure 2. Treatment application times for the two periodic application scenarios over a single cycle. Times are superimposed on the temperature sine curve. (Online version in colour.)

the abundances over all six cycles noted as 'infection pressure' estimates [36]).

At the start, the two periodic scenarios perform the best, as treating regardless of infection status keeps the abundance low. However, by the end of the second cycle, they are performing poorly and afterwards become the worst strategies for maintaining sea lice abundance. Responsive, mosaic and rotation all perform very similarly, with tight control of sea lice abundance over the first three cycles, with evidence of less effective control in the second year of the fourth cycle of production. During the fifth cycle, this breakdown in control becomes more apparent with total sea louse abundance doubling from three adult sea lice per salmon to six by the second half of the sixth cycle. Each production cycle shows elevated levels of sea lice in the second year of production, with a clear seasonal signal.

The *combination* scenario provides the best control overall, maintaining the sea louse abundance below the threshold of three adults per salmon throughout the six cycles. It also leads to the lowest overall infection pressure, with the motile sea louse abundance half (or less) of almost all the other scenarios (electronic supplementary material, table S1).

Across the scenarios that do not involve periodic intervention, approximately 17% of the population comprises adult sea lice during the initial part of the simulation (i.e. in cycle 1), with the rest divided roughly 43% to 36% between chalimus and pre-adult stages, respectively. However, in all but the *combination* scenarios, the proportion of adult sea lice increases by approximately 6% over the course of the six cycles. In the *combination* scenario, the overall proportion of adults is lower than in the rest of the scenarios (starting at roughly 16.5%), with the proportion increasing only marginally to 17.5% by cycle 6 (electronic supplementary material, figure S1). This suggests that there is a positive relationship between population-level resistance and the proportion of adults such that, when resistance occurs, the sea louse demography approaches an equilibrium, with higher proportions of adult sea lice relative to when there is no resistance. By contrast, when treatments are effective the proportion of adults is lower. The proportion of adults does not increase in the *combination* scenario, where treatments remained efficacious throughout the six cycle simulation. Our results suggest that the population structure of sea lice at a site might be a useful indicator of resistance.

4.2. Treatments

Sea lice abundance figures must be interpreted in concert with the number of treatment interventions used to achieve these levels. We saw in the previous section that *responsive*, *mosaic* and *rotation* all controlled sea louse abundance more effectively than the *periodic* strategies in the latter parts of the scenarios. However, figure 4 shows that they do so at the cost of greater numbers of treatment events. In addition, the number of treatment events for the first three strategies are increasing. The total treatment events in all these five cases are similar (electronic supplementary material, table S1). This is consistent with the fact that sea lice abundances for these three are similar (see the previous section), as are the predicted resistance profiles (see the next section).

The *combination* strategy performs best also by this measure. To maintain the sea lice abundance below the threshold, three treatments are required in the first five cycles, and four in the last cycle, giving a total of 19. Combination treatment events consist of synchronous application of both chemicals. This should be taken into consideration when making comparisons with other scenarios. From a farm management perspective, the strategy does not reduce chemical use as much as it first appears, though there are savings on time and effort required to administer treatments. Nonetheless, though we do not specify the exact concentrations of the two chemicals in our scenarios, even doubling the treatment event count (the worst case, where both chemicals are used at full dose) gives an event count substantially lower than the other strategies.

4.3. Resistance profiles

Sea louse abundances and treatment events are the most commonly recorded data from sea louse infestations on salmon farms. A benefit of the model is the ability to observe the evolution of resistance in the population (figure 5).

The *combination* strategy results in the slowest evolution of resistance, with less than 40% of the population resistant at the end of six production cycles. This is consistent with predictions [5], which state that the combination strategy will demonstrate the slowest emergence of resistance because, of all the strategies, it has the highest DTH. By exposing the population to two chemicals simultaneously, there is a high chance of killing any sea lice susceptible to either or both of the chemicals.

All of the other scenarios reach more than 95% resistance to both chemicals by the end of the six cycles. *Mosaic 30%* and *rotation* have similar resistance profiles to each other, and *responsive* reaches full resistance at a similar time. All three scenarios have over 70% resistance to both chemicals by production cycle 4. Both *periodic* application scenarios perform the most poorly, probably due to a large number of selection events that would not occur under a treatment trigger scenario.

The similarity of the *responsive*, *mosaic 30%* and *rotation* strategies bears some further discussion. The similarity could be because of a similar DTH. Treatments with different chemicals for *responsive* and *mosaic 30%* happen across generations and not within the same generation (i.e. the same sea louse is not exposed to two chemicals). This is also true for the earlier parts of the *rotation* strategy, where exceeding the treatment trigger threshold only happens infrequently. In the later parts of *rotation*, different chemicals may be

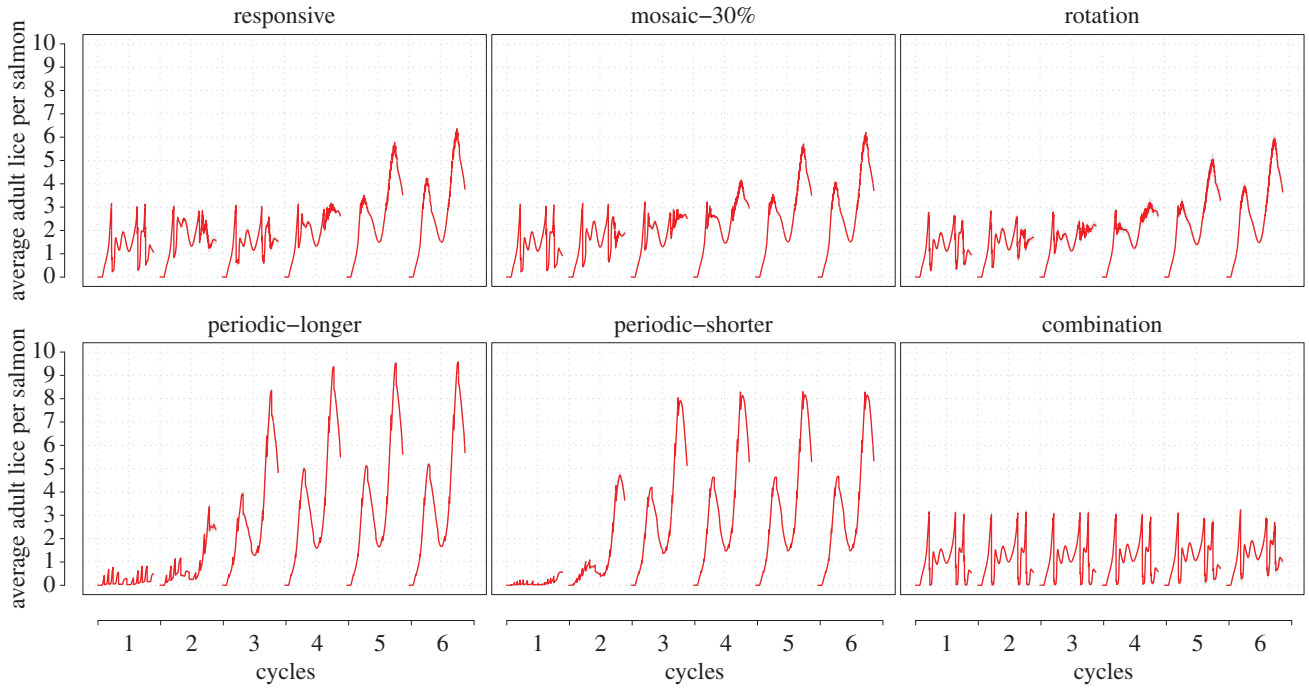

Figure 3. Abundance of adult sea lice (per salmon) over the six cycles. Six scenarios are shown. In all scenarios with thresholds, the threshold is three adult sea lice per salmon. The grey shaded areas show 95% CIs. (Online version in colour.)

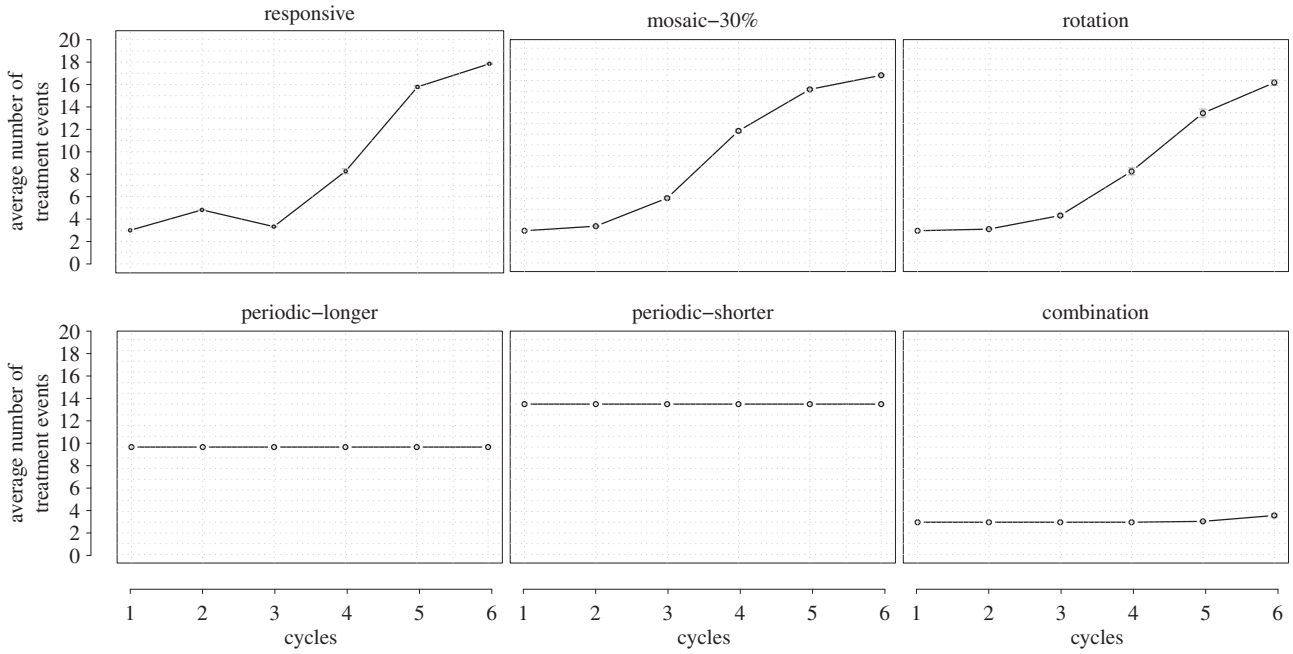

Figure 4. Average number of treatments required for each scenario. Bars indicate 95% CIs. (Online version in colour.)

applied to the same individuals, as treatment events are frequent, but by this time resistance to both chemicals is high. In the *mosaic 30%* scenario, an individual sea louse could be exposed to two chemicals moving from one cage to another, but adult dispersal rates are very small [32].

Similarities in the *rotation*, *responsive* and *mosaic* results have practical implications for farm management. Fish veterinary surgeons have often argued that it is important to give salmon farmers choices between treatments, because if they alternate between them then the selection pressure will be reduced. However, this does not appear to be the case, and having only one drug for several cycles, followed by another for the next several cycles, leaves you in no worse a position than if you had had access to both drugs from the beginning and alternated between cycles. In all scenarios, we see nearly 90% resistance in the population to both chemicals by the end of production cycle 5. This timing reflects what we have actually seen on farms, where it typically takes about a decade before treatments begin to notably lose their efficacy and another treatment must then be used [24,25]. As an interesting contrast, it is worth noting that, in some contexts, there has been no such emergence of resistance—e.g. the west coast of Canada [37]. The reason for this may be the presence of large wild populations of salmonids acting as refugia and fewer treatments being applied overall [18].

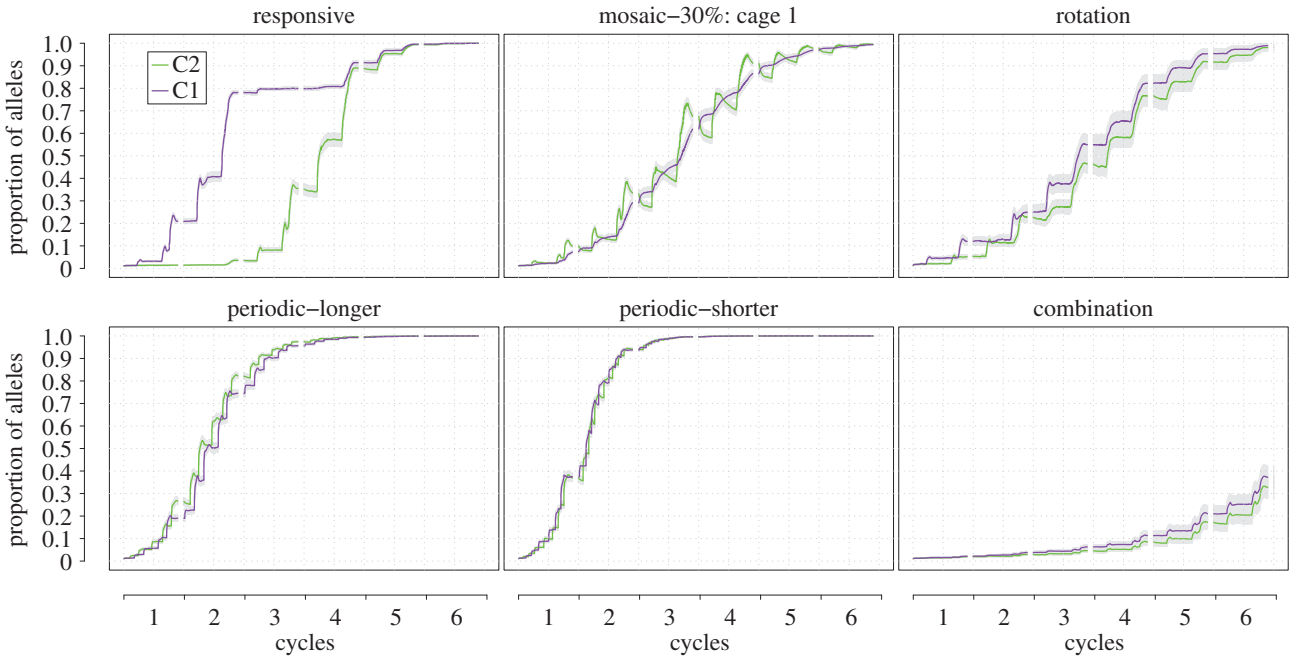

Figure 5. Resistance profiles for six scenarios over six production cycles. Grey shading shows 95% CIs. C2 has a 1% fitness cost associated with resistance so that chart lines do not overlap. (Online version in colour.)

4.4. Broader implications

Our results suggest that salmon farms can improve their management of sea lice resistance, thus maintaining better control over sea lice infestations, by applying chemicals in combination. This is consistent with studies of resistance in other domains, such as human diseases (e.g. HIV, leprosy, malaria and tuberculosis, reviewed in [4]) and terrestrial agricultural pests (reviewed in [5]). Currently, combination treatment strategies are not in wide use on salmon farms and many farms use some form of the *responsive alternation* strategy, applying a single chemical until it is no longer efficacious [7,10]. The salmon farming industry is now discussing combination strategies more extensively. Unfortunately, as there is widespread resistance to available chemicals, the starting point for most combination strategies will be less optimistic than our scenario, unless new drugs or compound classes are introduced.

There are many external factors that may limit farmers' ability to apply the combination strategy as outlined here. For example, differing costs of chemicals, availability of treatment equipment, when in the stocking cycle chemicals may be applied, and a lack of options. For example, several countries, such as Canada, have only one or two chemicals legally available to them [10]. Nonetheless, where possible, our results suggest that switching to a combination treatment strategy rather than relying heavily on one chemical per treatment could be beneficial for managing resistance, decreasing the number of treatments necessary, and reducing sea louse abundances.

5. Conclusion

The questions addressed in this study are close to the heart of the field of applied evolutionary biology, which has a large focus on rapid adaptation to novel stressors (such as pesticides). Inherent in evolutionary management is the idea that the long-term outcome is more important than the immediate one. In the case of sea lice, this translates to potentially having more expensive treatments that may be beneficial in the long term—for example, the combination scenario finishes in a better state than all the others; yet, the costs in the first three cycles could be higher. This is sometimes a difficult message for an industry that has more immediate economic or welfare concerns. ABMs could be used as educational tools to demonstrate to regulators, veterinary surgeons and farmers the long-term benefits of novel practices where the benefits are potentially delayed. For example, previously published models from our research group are available online and can be used by stakeholders to understand the impacts of different management strategies on sea louse population growth and resistance evolution [17,36].

6. Future work

This work has provided some insight into our questions about different treatment strategies in a limited setting. However, numerous questions and discussions arose out of the results. Below, we briefly mention some of the areas that we will be following up on next.

It is important to recognize that we have only explored using two bath treatment chemicals. Many other sea louse management strategies exist, such as: in-feed chemicals, which have a longer efficacy time; selective breeding for sea louse resistant salmon [38]; integrated pest management approaches such as coordinated regional management; use of mechanical methods such as cleaner fish predators or lasers [3,39,40]; and maintenance of wild salmonid populations to act as 'chemical refugia' for sea lice [9,18]. We would like to incorporate these other chemical and non-chemical options into the model to investigate how they can be used together for greatest effect. We predict that adding more treatment options will, in general, slow down the evolution of resistance. This will be especially true if there are techniques available that are less susceptible to resistance, such as the mechanical methods.

There are a number of potential complexities in the genetic architecture of resistance that we would like to incorporate into future versions of this model. For example, cross-resistance and/or multiple drug resistance mechanisms between treatment chemicals may occur [10], which could decrease the time taken to develop resistance; resistance genes may have epistatic or pleiotropic effects that alter fitness and/or resistance; and there are many gender differences in responses to chemical stimuli [30,41,42] and even to the structure and rate of evolution of male and female genomes [43]. We have modelled resistance with a single gene with codominant alleles, and, in some cases, this is most likely to be the correct mechanism [28]. However, resistance to other chemicals will have different and possibly polygenic mechanisms. These effects will change the relative strengths and weaknesses of the strategies we have investigated.

In our current model, there is no fitness cost for the resistance mutation; a result that is generally borne out by previous research, e.g. [42]. However, if there was a fitness cost associated with resistance, it may alter our results. Because there is no fitness cost in our scenarios, the time period between selection events has little influence on evolutionary dynamics. For example, in the *responsive* scenario, chemical 1 is subject to selection for most of the first two production cycles and then is rested—not used—for almost two cycles. In this study, the rest period does not result in any substantial change in resistance. However, if there was a fitness cost of resistance, this could result in a reduction of resistance alleles, thereby improving the performance of the responsive strategy. In a similar way, fitness costs may also have a substantial effect on the *rotation* strategy though in that case through many smaller rest periods between selection events on the two chemicals. The presence of a refugia could extend this effect by making the selection events against a particular portion of the population even less frequent; possibly with enough exposure to a sufficiently large refugia and the right fitness cost, the evolution of resistance could be negated entirely.

The ABM framework presented here is flexible enough that it can incorporate the complex genetic mechanisms and treatment applications described above. Our current results provide a valuable baseline from which to compare these proposed models.

Code and materials

The model code is available at https://github.com/gmcewan/SalmonFarmTreatmentStrategy. Also included are a README file on how to run the code and the configuration files that we used for this study.

Authors' contributions. M.L.G. conceived of the study. G.F.M. implemented the model, ran the scenarios and created most of the figures. D.L.B. and C.W.R. wrote up the results and discussion analysis. C.W.R. coordinated the study. M.D.F. provided expert advice on parametrizing the model. All authors helped draft and edit the manuscript. All authors gave final approval for publication.

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References

1. Michigan State University. *Arthropod pesticide resistance database*. http://www.pesticideresistance.org/index.php (accessed 11 Oct 2016).
2. Brattsten LB, Holyoke CW, Leeper JR, Raffa KF. 1986 Insecticide resistance: challenge to pest management and basic research. *Science* **231**, 1255–1260. (doi:10.1126/science.231.4743.1255)
3. Hillocks RJ. 2012 Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Prot*. **31**, 85–93. (doi:10.1016/j.cropro.2011.08.008)
4. Carroll SP, Jørgensen PS, Kinnison MT, Bergstrom CT, Denison RF, Gluckman P, Smith TB, Strauss SY, Tabashnik BE. 2014 Applying evolutionary biology to address global challenges. *Science* **346**, 1245993. (doi:10.1126/science.1245993)
5. REX Consortium. 2013 Heterogeneity of selection and the evolution of resistance. *Trends Ecol. Evol*. **28**, 110–118. (doi:10.1016/j.tree.2012.09.001)
6. Read AF, Day T, Huijben S. 2011 The evolution of drug resistance and the curious orthodoxy of aggressive chemotherapy. *Proc. Natl Acad. Sci. USA* **108**(Suppl. 2), 10 871–10 877. (doi:10.1073/pnas.1100299108)
7. Denholm I, Devine GJ, Horsberg TE, Sevatdal S, Fallang A, Nolan DV, Powell R. 2002 Analysis and management of resistance to chemotherapeutants in salmon lice, *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Pest Manage. Sci*. **58**, 528–536. (doi:10.1002/ps.482)
8. Igboeli OO, Burka JF, Fast MD. 2014 *Lepeophtheirus salmonis*: a persisting challenge for salmon aquaculture. *Anim. Front*. **4**, 22–32. (doi:10.2527/af.2014-0004)
9. Murray AG. 2011 A simple model to assess selection for treatment-resistant sea lice. *Ecol. Modell*. **222**, 1854–1862. (doi:10.1016/j.ecolmodel.2011.03.016)
10. Aaen SM, Helgesen KO, Bakke MJ, Kaur K, Horsberg TE. 2015 Drug resistance in sea lice: a threat to salmonid aquaculture. *Trends Parasitol*. **31**, 72–81. (doi:10.1016/j.pt.2014.12.006)
11. Beveridge MCM, Thilsted SH, Phillips MJ, Metian M, Troell M, Hall SJ. 2013 Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. *J. Fish Biol*. **83**, 1067–1084. (doi:10.1111/jfb.12187)
12. Food and Agriculture Organization of the United Nations, Fisheries and Aquaculture Department. 2014 *The state of world fisheries and aquaculture: opportunities and challenges*. Rome, Italy: Food and Agriculture Organization of the United Nations.
13. Adams T, Black K, MacIntyre C, MacIntyre I, Dean R. 2012 Connectivity modelling and network analysis of sea lice infection in Loch Fyne, west coast of Scotland. *Aquac. Environ. Interact*. **3**, 51–63. (doi:10.3354/aei00052)
14. Groner ML, McEwan GF, Rees EE, Gettinby G, Revie CW. 2016 Quantifying the influence of salinity and temperature on the population dynamics of a marine ectoparasite. *Can. J. Fish. Aquat. Sci*. **73**, 1281–1291. (doi:10.1139/cjfas-2015-0444)
15. Costello MJ. 2009 The global economic cost of sea lice to the salmonid farming industry. *J. Fish Dis*. **32**, 115–118. (doi:10.1111/j.1365-2761.2008.01011.x)
16. Krkošek M, Gottesfeld AS, Proctor B, Rolston D, Carr-Harris C, Lewis MA. 2007 Effects of host migration, diversity and aquaculture on sea lice threats to Pacific salmon populations. *Proc. R. Soc. B* **274**, 3141–3149. (doi:10.1098/rspb.2007.1122)
17. Tully O, Nolan DT. 2002 A review of the population biology and host–parasite interactions of the sea louse *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Parasitology* **124**, 165–182. (doi:10.1017/S0031182002001889)
18. McEwan GF, Groner ML, Fast MD, Gettinby G, Revie CW. 2015 Using agent-based modelling to predict the role of wild refugia in the evolution of resistance

of sea lice to chemotherapeutants. *PLoS ONE* **10**, e0139128. (doi:10.1371/journal.pone.0139128)
19. Hastie LC *et al.* 2013 Prevalence and infection intensity of sea lice (*Lepeophtheirus salmonis*) on Atlantic salmon (*Salmo salar*) host is reduced by the non-host compound 2-aminoacetophenone. *Aquaculture* **410-411**, 179–183. (doi:10.1016/j.aquaculture.2013.06.035)
20. Stratonovitch P, Elias J, Denholm I, Slater R, Semenov MA. 2014 An individual-based model of the evolution of pesticide resistance in heterogeneous environments: control of *Meligethes aeneus* population in oilseed rape crops. *PLoS ONE* **9**, e115631. (doi:10.1371/journal.pone.0115631)
21. DeAngelis DL, Mooij WM. 2005 Individual-based modeling of ecological and evolutionary processes. *Annu. Rev. Ecol. Evol. Syst.* **36**, 147–168. (doi:10.1146/annurev.ecolsys.36.102003.152644)
22. REX Consortium. 2010 The skill and style to model the evolution of resistance to pesticides and drugs. *Evol. Appl.* **3**, 375–390. (doi:10.1111/j.1752-4571.2010.00124.x)
23. Grimm V *et al.* 2006 A standard protocol for describing individual-based and agent-based models. *Ecol. Modell.* **198**, 115–126. (doi:10.1016/j.ecolmodel.2006.04.023)
24. Lees F, Baillie M, Gettinby G, Revie CW. 2008 The efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on farmed Atlantic salmon (*Salmo salar* L) in Scotland, 2002–2006. *PLoS ONE* **3**, e1549. (doi:10.1371/journal.pone.0001549)
25. Jones PG, Hammell KL, Dohoo IR, Revie CW. 2012 Effectiveness of emamectin benzoate for treatment of *Lepeophtheirus salmonis* on farmed Atlantic salmon *Salmo salar* in the Bay of Fundy, Canada. *Dis. Aquat. Org.* **102**, 53–64. (doi:10.3354/dao02517)
26. Jones PG, Hammell KL, Gettinby G, Revie CW. 2013 Detection of emamectin benzoate tolerance emergence in different life stages of sea lice, *Lepeophtheirus salmonis*, on farmed Atlantic salmon, *Salmo salar* L. *J. Fish Dis.* **36**, 209–220. (doi:10.1111/jfd.12022)
27. Groner ML, Gettinby G, Stormoen M, Revie CW, Cox R. 2014 Modelling the impact of temperature-induced life history plasticity and mate limitation on the epidemic potential of a marine ectoparasite. *PLoS ONE* **9**, e88465. (doi:10.1371/journal.pone.0088465)
28. Kaur K, Jansen PA, Aspehaug VT, Horsberg TE. 2016 Phe362Tyr in AChE: a major factor responsible for azamethiphos resistance in *Lepeophtheirus salmonis* in Norway. *PLoS ONE* **11**, e0149264. (doi:10.1371/journal.pone.0149264)
29. ffrench-Constant RH. 2013 The molecular genetics of insecticide resistance. *Genetics* **194**, 807–815. (doi:10.1534/genetics.112.141895)
30. Sutherland BJG *et al.* 2015 Transcriptomic responses to emamectin benzoate in Pacific and Atlantic Canada salmon lice *Lepeophtheirus salmonis* with differing levels of drug resistance. *Evol. Appl.* **8**, 133–148. (doi:10.1111/eva.12237)
31. Revie CW, Gettinby G, Treasurer JW, Wallace C. 2003 Identifying epidemiological factors affecting sea lice *Lepeophtheirus salmonis* abundance on Scottish salmon farms using general linear models. *Dis. Aquat. Organ.* **57**, 85–95. (doi:10.3354/dao057085)
32. Connors BM, Lagasse C, Dill LM. 2011 What's love got to do with it? Ontogenetic changes in drivers of dispersal in a marine ectoparasite. *Behav. Ecol.* **22**, 588–593. (doi:10.1093/beheco/arr024)
33. Glynn D *et al.* 2013 *Chemical residue in Irish farmed fnfish, 2011*. Marine Institute. http://oar.marine.ie/handle/10793/876 (accessed 13 Sep 2016).
34. Berntssen MHG, Valdersnes S, Lunestad BT, Hatlen B, Alm M, Waagbø R, Buttle L. 2014 Residue levels of enrofloxacin and ciprofloxacin in processed animal by-products used in Atlantic salmon feeds and their long-term carry-over to the edible part of the fish. *Aquacult. Nutr.* **20**, 712–721. (doi:10.1111/anu.12121)
35. Salama N, Murray A. 2011 Farm size as a factor in hydrodynamic transmission of pathogens in aquaculture fish production. *Aquac. Environ. Interact.* **2**, 61–74. (doi:10.3354/aei00030)
36. Robbins C, Gettinby G, Lees F, Baillie M, Wallace C, Revie CW. 2010 Assessing topical treatment interventions on Scottish salmon farms using a sea lice (*Lepeophtheirus salmonis*) population model. *Aquaculture* **306**, 191–197. (doi:10.1016/j.aquaculture.2010.05.006)
37. Saksida SM, Morrison D, Revie CW. 2010 The efficacy of emamectin benzoate against infestations of sea lice, *Lepeophtheirus salmonis*, on farmed Atlantic salmon, *Salmo salar* L., in British Columbia. *J. Fish Dis.* **33**, 913–917. (doi:10.1111/j.1365-2761.2010.01192.x)
38. Jones CS, Lockyer AE, Verspoor E, Secombes CJ, Noble LR. 2002 Towards selective breeding of Atlantic salmon for sea louse resistance: approaches to identify trait markers. *Pest Manage. Sci.* **58**, 559–568. (doi:10.1002/ps.511)
39. Groner ML, Cox R, Gettinby G, Revie CW. 2013 Use of agent-based modelling to predict benefits of cleaner fish in controlling sea lice, *Lepeophtheirus salmonis*, infestations on farmed Atlantic salmon, *Salmo salar* L. *J. Fish Dis.* **36**, 195–208. (doi:10.1111/jfd.12017)
40. Treasurer JW. 2002 A review of potential pathogens of sea lice and the application of cleaner fish in biological control. *Pest Manage. Sci.* **58**, 546–558. (doi:10.1002/ps.509)
41. Igboeli OO, Purcell SL, Wotton H, Poley J, Burka JF, Fast MD. 2013 Immunostimulation of *Salmo salar* L., and its effect on *Lepeophtheirus salmonis* (Krøyer) P-glycoprotein mRNA expression following subsequent emamectin benzoate exposure. *J. Fish Dis.* **36**, 339–351. (doi:10.1111/jfd.12063)
42. Igboeli OO, Burka JF, Fast MD. 2011 Sea lice population and sex differences in P-glycoprotein expression and emamectin benzoate resistance on salmon farms in the Bay of Fundy, New Brunswick, Canada. *Pest Manage. Sci.* **70**, 905–914. (doi:10.1002/ps.3620)
43. Poley JD, Sutherland BJG, Jones SRM, Koop BF, Fast MD. 2016 Sex-biased gene expression and sequence conservation in Atlantic and Pacific salmon lice (*Lepeophtheirus salmonis*). *BMC Genomics* **17**, 483. (doi:10.1186/s12864-016-2835-7)